Remarks/Arguments

The foregoing amendments to the claims are of formal nature, and do not add new matter. Applicants have amended the title to better describe the claimed invention. The foregoing amendments to the specification delete all embedded hyperlinks and any minor errors have been amended.

Claims 119-124 are pending in this application and are rejected on various grounds. Pending claims have been amended to remove references to "Figures" and to more clearly claim what the Applicants consider their invention. Claims 122 and 124 have been canceled without prejudice or disclaimer. Accordingly, Claims 119-121 and 123 are currently pending in this application and rejections to these claims are respectfully traversed.

Priority

The nucleic acid sequence of SEQ ID NO: 228 and the polypeptide of SEQ ID NO: 229 were first disclosed in U.S. provisional application 60/096768, filed **August 17, 1998.**

Applicants rely on the 'gene amplification' assay (Example 143) for patentable utility of the subject matter relating to claims 119-123. This utility was first disclosed in the US Provisional Application 60/141,037, filed June 23, 1999, priority for which has been claimed in this application. Hence, the present application is at least entitled to an effective filing date of **June 23, 1999** based on results of the 'gene amplification' assay.

Further, Applicants rely on the 'chondrocyte proliferation' assay (Example 153) for patentable utility of the subject matter relating to claims 119-123. This utility was first disclosed in International Application PCT/US00/08439, filed March 30, 2000, priority for which has been claimed in the instant application. Hence, the present application is at least entitled to an effective filing date of **March 30, 2000** based on results of the 'chondrocyte proliferation' assay.

The Examiner has acknowledged utility for the claimed protein and antibodies based on the chondrocyte redifferentiation assay. As per the Examiner's request, Applicants have provided a copy of the relevant portion of the PCT application, which is identical to the instant application, that contains the chondrocyte redifferentiation assay for priority determination.

The Examiner further indicates that the gene amplification assay was not found to be enabling as required by the 35 U.S.C.§ 112, first paragraph. The Examiner indicates that the

amplification was "mild" and also says that "a slight amplification of a gene does not necessarily mean overexpression in a cancer tissue, but can merely be an indication that the cancer tissue is aneuploid". The Examiner quotes an exemplary reference like Sen and concludes that "the data was not corrected for aneuploidy". The Examiner further quotes Haynes *et al.,* Pennica *et al.* and Konopka *et al.* to show that "an increase in nucleic acid copy number is not predictive of a similar association for protein." For the reasons provided below, Applicants respectfully disagree.

Regarding the rejection on lack of correction of data based on aneuploidy, Applicants submit that, as noted by the Examiner and the Sen article, aneuploid tissues are cancerous or pre-cancerous. The present invention is directed to proteins and antibodies useful in the detection of cancer, irrespective of the mechanism by which gene amplification occurs. Even if aneuploid tissues were to predict a propensity for cancer, the instant proteins and antibodies are still useful as diagnostic tools. Applicants have included a declaration by Avi Ashkenazi, Ph.D., a co-inventor of this application, who says that:

> "An increase in gene copy number can result not only from intrachromosomal changes but also from chromosomal aneuploidy. It is important to understand that detection of gene amplification can be used for cancer diagnosis even if the determination includes measurement of chromosomal aneuploidy. Indeed, as long as a significant difference relative to normal tissue is detected, it is irrelevant if the signal originates from an increase in the number of gene copies per chromosome and/or an abnormal number of chromosomes."

Further, regarding the Examiner's rejection that "an increase in nucleic acid copy number is not predictive of a similar association for protein," Applicants first argue the teachings of the articles cited by the Examiner and provide exemplary articles to support their position that, it is more likely than not that amplified DNA results in amplified protein levels, barring certain exceptions.

Pennica *et al.* teaches that "An analysis of *WISP*-1 gene amplification and expression in human colon tumors **showed a correlation between DNA amplification and over-expression**, ... In contrast, *WISP*-2 DNA was amplified in colon tumors, but its mRNA expression was significantly reduced in the majority of tumors compared with expression in normal colonic mucosa from the same patient." (Emphasis added). The Applicants draw attention to Pennica's

showing that "a correlation between DNA amplification and over-expression exists for the *WISP*-1 gene"in 84% of the tumors examined. While Pennica discloses a lack of correlation for the *WISP*-2 gene, Pennica teaches nothing regarding such a lack of correlation in genes in general. That is, Pennica's teachings are specific for the *WISP* family of genes, and are not directed to genes in general. The Utility Guidelines requires that for a *prima facie* showing of lack of utility, the Examiner has to provides evidence that it is **more likely than not** that a lack of correlation between protein expression and gene amplification exists, in general. Accordingly, Applicants respectfully submit that Pennica teaches nothing of the correlation between gene amplification and polypeptide over-expression in general.

Regarding the Examiner's rejection based on Konopka *et al.*, again, Applicants respectfully submit that the Examiner has generalized a result pertaining to merely **one** gene, the *abl* gene, to cover all genes in general. Konopka does not disclose any generalized teaching about the correlation between protein expression and gene amplification. Applicants submit that the Konopka reference is not sufficient to establish such a *prima facie* showing of lack of utility based on the results with the *abl* gene alone. Thus, the combined teachings of Pennica and Konopka are not directed towards genes in general but to single genes or genes within a family and thus, their teachings have been misrepresented in this rejection.

Regarding Haynes, the Examiner says that "Haynes *et al.* studied 80 proteins... and found no strong correlation between proteins and transcript levels." Applicants respectfully traverse and point out that, on the contrary, Haynes teaches that "**there was a general trend** but no strong correlation between protein [expression] and transcript levels" (Emphasis added). Haynes studied 80 *yeast* proteins to show that "protein levels cannot be **accurately** predicted from the level of the corresponding mRNA transcript" (Emphasis added) (see page 1863, paragraph 2.1, last line). For example, in Figure 1, there is a positive correlation between mRNA and protein amongst **most** of the 80 yeast proteins studied but the correlation is "not linear" and hence, "one cannot **accurately** predict protein levels from mRNA levels." In fact, very few data points deviated or scattered away from the expected normal or showed a lack of correlation between mRNA: protein levels. Thus, the Haynes data meets the "more likely than not standard" and shows that a positive correlation exists between mRNA and protein.

In conclusion, the Examiner has not shown that a lack of correlation between gene amplification: polypeptide over-expression, as observed for the *WISP-2* or the *abl* genes, is typical. In fact, contrary to what the Examiner contends, the art indicates that, if a gene is amplified in cancer, it is **more likely than not** that the encoded protein will be expressed at an elevated level. As noted even in Pennica *et al.*, a correlation between DNA amplification: polypeptide over-expression was observed in the case of *WISP-1* and similarly, in Haynes *et al.*, **most genes** showed a correlation between increased mRNA : translated protein. Since the standard is not absolute certainty, a *prima facie* showing of lack of utility has not been made in this instance.

It is "more likely than not" for amplified genes to have increased mRNA and protein levels

Applicants submit further exemplary articles to show that, contrary to what the Examiner asserts, just as in Haynes, the art indicates that, generally, if a gene is amplified in cancer, it is **more likely than not** that the encoded protein will be expressed at an elevated level. For example, Ornoft *et al.* (Mol. and Cell. Proteomics, 2002, Vol.1, pages 37-45) studied transcript levels of 5600 genes in malignant bladder cancers many of which were linked to the gain or loss of chromosomal material using an array-based method. Ornoft *et al.* showed that there was a gene dosage effect and taught that "in general (18 of 23 cases) chromosomal areas with more than 2-fold gain of DNA showed a corresponding increase in mRNA transcripts" (see column 1, abstract). In addition, Hyman *et al.* (Cancer Res., 2002, Vol. 62, pages 6240-45) showed, using CGH analysis and cDNA microarrays which compared DNA copy numbers and mRNA expression of over 12,000 genes in breast cancer tumors and cell lines, that there was "evidence of a prominent global influence of copy number changes on gene expression levels." (see page 6244, column 1, last paragraph). Additional supportive teachings were also provided by Pollack *et al.*, (PNAS, 2002, Vol. 99, pages 12963-12968) who studied a series of primary human breast tumors and showed that "...62% of highly amplified genes show moderately or highly elevated expression, and DNA copy number influences gene expression across a wide range of DNA copy number alterations (deletion, low-, mid- and high-level amplification), and that on average, a 2-fold change in DNA copy number is associated with a corresponding 1.5-fold change in

mRNA levels." Thus, these articles collectively teach that in general, gene amplification increases mRNA expression.

In addition, enclosed is a Declaration by Dr. Polakis, principal investigator of the Tumor Antigen Project of Genentech, Inc., the assignee of the present application to show that mRNA expression correlates well with protein levels, in general. As Dr. Polakis explains, the primary focus of the microarray project was to identify tumor cell markers useful as targets for both the diagnosis and treatment of cancer in humans. The scientists working on the project extensively rely on results of microarray experiments in their effort to identify such markers. As Dr. Polakis explains, using microarray analysis, Genentech scientists have identified approximately 200 gene transcripts (mRNAs) that are present in human tumor cells at significantly higher levels than in corresponding normal human cells. To date, they have generated antibodies that bind to about 30 of the tumor antigen proteins expressed from these differentially expressed gene transcripts and have used these antibodies to quantitatively determine the level of production of these tumor antigen proteins in both human cancer cells and corresponding normal cells. Having compared the levels of mRNA and protein in both the tumor and normal cells analyzed, they found a very good correlation between mRNA and corresponding protein levels. Specifically, in approximately 80% of their observations they have found that increases in the level of a particular mRNA correlates with changes in the level of protein expressed from that mRNA. While the proper legal standard is to show that the existence of correlation between mRNA and polypeptide levels is more likely than not, the showing of approximately 80% correlation for the molecules tested in the Polakis Declaration greatly exceed this legal standard. Based on these experimental data and his vast scientific experience of more than 20 years, Dr. Polakis states that, for human genes, increased mRNA levels typically correlate with an increase in abundance of the encoded protein. He further confirms that "it remains a central dogma in molecular biology that increased mRNA levels are predictive of corresponding increased levels of the encoded protein."

Taken together, although there are some examples in the scientific art that do not fit within the central dogma of molecular biology, that there is a correlation between polypeptide and mRNA levels, these instances are exceptions rather than the rule. In the vast majority of amplified genes, the teachings in the art, as exemplified by Orntoft *et al.*, Hyman *et al.*, Pollack *et al.*, and the Polakis declaration, overwhelmingly show that gene amplification influences gene

expression at the mRNA and protein levels. Thus, one of skill in the art would reasonably expect in this instance, based on the amplification data for the PRO1111 gene, that the PRO1111 protein is concomitantly overexpressed. Thus, Applicants submit that the PRO1111 proteins have utility in the diagnosis of cancer and based on such a utility, one of skill in the art would know exactly how to use the protein and its antibodies for the diagnosis of cancer.

Claimed proteins would have diagnostic utility even if the protein were not overexpressed

Even assuming *arguendo* that, there is no correlation between gene amplification and increased mRNA/protein expression for PRO1111, which Applicants submit is not true, a polypeptide encoded by a gene that is amplified in lung or colon squamous cell carcinomas or adenocarcinomas would **still** have a credible, specific and substantial utility. In support, Applicants submit a Declaration by Avi Ashkenazi, Ph.D., an expert in the field of cancer biology and an inventor of the instant application. Dr. Avi Ashkenazi's Declaration explains that:

> even when amplification of a cancer marker gene does not result in significant over-expression of the corresponding gene product, this very absence of gene product over-expression still provides significant information for cancer diagnosis and treatment. Thus, if over-expression of the gene product does not parallel gene amplification in certain tumor types but does so in others, then parallel monitoring of gene amplification and gene product over-expression enables more accurate tumor classification and hence better determination of suitable therapy. In addition, absence of over-expression is crucial information for the practicing clinician. If a gene is amplified but the corresponding gene product is not over-expressed, the clinician accordingly will decide not to treat a patient with agents that target that gene product.

Applicants thus submit that simultaneous testing of gene amplification and gene product over-expression enables more accurate tumor classification, even if the gene-product, the protein, is not over-expressed. This leads to better determination of a suitable therapy. Further, as explained in Dr. Ashkenazi's Declaration, absence of over-expression of the protein itself is crucial information for the practicing clinician. If a gene is amplified in a tumor, but the corresponding gene product is not over-expressed, the clinician need not treat a patient with agents that target that gene product. This not only saves money, but further prevents unnecessary exposure of the patient to the side effects of gene product targeted agents.

This is further supported by the teachings of the attached article by Hanna and Mornin. The article teaches that the HER-2/neu gene has been shown to be amplified and/or over-expressed in 10%-30% of invasive breast cancers and in 40%-60% of intraductal breast carcinoma. Further, the article teaches that diagnosis of breast cancer includes testing both the amplification of the HER-2/neu gene (by FISH) as well as the over-expression of the HER-2/neu gene product (by IHC). Even when the protein is not over-expressed, the assay relying on both tests leads to a more accurate classification of the cancer and a more effective treatment of it.

In conclusion, Applicants have demonstrated a credible, specific and substantial asserted utility for the PRO1111 polypeptide based on the gene amplification results for the nucleic acid, for example, in detecting over-expression or absence of expression of PRO1111. In fact, the art also indicates that, if a gene is amplified in cancer, it is **more likely than not** that the encoded protein will also be expressed at an elevated level. Based on these discussions, one skilled in the art, at the time the application was filed, would know how to use the claimed polypeptides, without undue experimentation.

Thus, Applicants have demonstrated utility for the PRO1111 polypeptide and its antibodies based on the gene amplification assay and thus, Applicants request that the Examiner reconsider the priority date for the present application based on the present arguments.

Claim Rejections – 35 U.S.C. § 112, second paragraph

Claims 119-124 are rejected under 35 U.S.C. §112, second paragraph for being indefinite for reciting "specifically binds". Applicants respectfully traverse these rejections.

Without acquiescing to the propriety of this rejection and solely in the interest of expedited prosecution in this case, Applicants have canceled claim 124 and therefore, this rejection is moot. Further, Applicants have amended claim 119 to recite "specifically binds" and submit that the art-recognized meaning of "specifically binds" is that the antibody binds to a particular antigen and does not significantly cross-react with another antigen.

Accordingly, this term is definite and this rejection should be withdrawn.

Claim Rejections – 35 U.S.C. §102

1) Claims 119-122 and 124 are rejected under 35 U.S.C. §102(a) or (b) as being anticipated by Jacobs, WO 99/50405 (pub 10/7/1999).

2) Claims 119-124 are rejected under 35 U.S.C. §102(e) as being anticipated by Shimkets, USPN 6,689,866 or US patent Pub US2003/0054514 or US patent Pub US2003/0003532 (dated 3/8/00).

As discussed above under the section on priority, Applicants are at least entitled to an effective filing date of **June 23, 1999** based on the results of the 'gene amplification' assay for Claims 119-122 and 124. Therefore, neither Jacobs nor Shimkets are prior art. Therefore, these rejections should be withdrawn.

Claim Rejections – 35 U.S.C. §103(a)

1) Claims 119-120 and 123-124 are rejected under 35 U.S.C. §103(a) as being obvious over any one loci AI769814, AI435407, AI470931 or T15752 in view of Sibson et al.

2) Claims 121-122 are rejected under 35 U.S.C. §103(a) as being obvious over any one loci AI769814, AI435407, AI470931 or T15752 in view of Sibson et al. and further in view of USPN 5,565,332 (Hoogenboom) in the case of claim 121, or in view of USPN 4,946,778 (Ladner) in the case of claim 122.

Again, as discussed under priority, Applicants are at least entitled to an effective filing date of **August 17, 1998** for the protein and nucleic acid sequences of SEQ ID NOs: 229 and 228, respectively and further, Applicants are at least entitled to an effective filing date of **June 23, 1999** based on the results of the 'gene amplification' assay. Further, Applicants submit that ESTs AI769814, AI435407, AI470931 or T15752 are not enabling disclosures since they do not provide any utility. Therefore, Applicants submit that ESTs AI769814, AI435407, AI470931 or T15752 are not prior art.

Since the primary references are not 103(a) references, and Sibson or Hoogenboom or Ladner do not teach SEQ ID NOs: 229 and 228 of the instant application, this rejection falls and should be withdrawn.

3) Claim 123 is rejected under 35 U.S.C. §103(a) as being obvious over Jacobs, WO99/50405 (dated pub 10/7/1999) as applied to claims 120-122 and 124 above.

As discussed above, Jacobs is not prior art since Applicants are at least entitled to an effective filing date of 10/7/1999. Accordingly, this rejection should be withdrawn.

The present application is believed to be in *prima facie* condition for allowance, and an early action to that effect is respectfully solicited.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 08-1641 (Attorney Docket No.: 39780-2730P1C18). Please direct any calls in connection with this application to the undersigned at the number provided below.

Respectfully submitted,

Date: November 1, 2004

Daphne Reddy

Daphne Reddy
Reg. No. 53,507

HELLER EHRMAN WHITE & McAULIFFE LLP
Customer No. 35489
275 Middlefield Road
Menlo Park, California 94025
Telephone: (650) 324-7000
Facsimile: (650) 324-0638
2067819

EXAMPLE 158: Detection of PRO Polypeptides That Affect Glucose or FFA Uptake by Primary Rat Adipocytes (Assay 94)

This assay is designed to determine whether PRO polypeptides show the ability to affect glucose or FFA uptake by adipocyte cells. PRO polypeptides testing positive in this assay would be expected to be useful for the therapeutic treatment of disorders where either the stimulation or inhibition of glucose uptake by adipocytes would be beneficial including, for example, obesity, diabetes or hyper- or hypo-insulinemia.

In a 96 well format, PRO polypeptides to be assayed are added to primary rat adipocytes, and allowed to incubate overnight. Samples are taken at 4 and 16 hours and assayed for glycerol, glucose and FFA uptake. After the 16 hour incubation, insulin is added to the media and allowed to incubate for 4 hours. At this time, a sample is taken and glycerol, glucose and FFA uptake is measured. Media containing insulin without the PRO polypeptide is used as a positive reference control. As the PRO polypeptide being tested may either stimulate or inhibit glucose and FFA uptake, results are scored as positive in the assay if greater than 1.5 times or less than 0.5 times the insulin control.

The following PRO polypeptides tested positive as stimulators of glucose and/or FFA uptake in this assay: PRO1114, PRO1007, PRO1066, PRO848, PRO1182, PRO1198, PRO1192, PRO1271, PRO1375 and PRO1387.

The following PRO polypeptides tested positive as inhibitors of glucose and/or FFA uptake in this assay: PRO1184, PRO1360, PRO1309, PRO1154, PRO1181, PRO1186, PRO1160 and PRO1384.

EXAMPLE 159: Chondrocyte Re-differentiation Assay (Assay 110)

This assay shows that certain polypeptides of the invention act to induce redifferentiation of chondrocytes, therefore, are expected to be useful for the treatment of various bone and/or cartilage disorders such as, for example, sports injuries and arthritis. The assay is performed as follows. Porcine chondrocytes are isolated by overnight collagenase digestion of articulary cartilage of metacarpophalangeal joints of 4-6 month old female pigs. The isolated cells are then seeded at 25,000 cells/cm^2 in Ham F-12 containing 10% FBS and 4 μg/ml gentamycin. The culture media is changed every third day and the cells are then seeded in 96 well plates at 5,000 cells/well in 100μl of the same media without serum and 100 μl of the test PRO polypeptide, 5 nM staurosporin (positive control) or medium alone (negative control) is added to give a final volume of 200 μl/well. After 5 days of incubation at 37°C, a picture of each well is taken and the differentiation state of the chondrocytes is determined. A positive result in the assay occurs when the redifferentiation of the chondrocytes is determined to be more similar to the positive control than the negative control.

The following polypeptide tested positive in this assay: PRO1282, PRO1310, PRO619, PRO943, PRO820, PRO1080, PRO1016, PRO1007, PRO1056, PRO791, PRO1111, PRO1184, PRO1360, PRO1309, PRO1107, PRO1132, PRO1131, PRO848, PRO1181, PRO1186, PRO1159, PRO1312, PRO1192 and PRO1384.